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RAPID COMMUNICATION

J Neurophysiol
88: 523–527, 2002; 10.1152/jn.00838.2001.

Paradoxical Anti-Epileptic Effects of a GluR5 Agonist of Kainate Receptors

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Received 15 October 2001; accepted in final form 24 January 2002

Khalilov, Ilgam, June Hirsch, Rosa Cossart, and Yehezkel Ben-Ari. Paradoxical anti-epileptic effects of a GluR5 agonist of kainate receptors. *J Neurophysiol* 88: 523–527, 2002; 10.1152/jn.00838.2001. Kainate generates in adult hippocampal neurons a seizure but also a massive excitation of interneurons and a dramatic increase of the inhibitory drive that impinges on principal cells. This "overinhibition" is largely mediated by GluR5-containing kainate receptors that are enriched on interneurons. Here, using the neonatal intact hippocampus in vitro and the triple chamber, we first show that this mechanism is fully operative in neonatal neurons. We then report that application to one hippocampus of (*RS*)-2-amino-3-(5-tert-butyl-3-hydroxy-4-isoxazolyl)propionic acid—a relatively selective agonist of GluR5 containing kainate receptors—depresses the propagation of seizure generated in the opposite hippocampus by a convulsive agent. We conclude that the selective excitation of interneurons by GluR5-containing kainate receptor agonists opens a new therapeutic approach for the epilepsies.

INTRODUCTION

Systemic or intracerebral administration of kainate—an excitatory amino acid extracted from sea weeds—generates a seizure and brain-damage syndrome that mimics several central properties of human temporal lobe epilepsy (Ben-Ari 1985; Nadler 1981). Studies using kainate and kainate analogues in vivo and in slice preparations have shown that kainate generates seizures in the CA3 region—by far the most susceptible brain region to the adverse effect of kainate (Ben-Ari and Gho 1988; Robinson and Deadwyler 1981). These events then propagate to the CA1 region and from there to other limbic structures leading to the occurrence of recurrent limbic seizures and a status epilepticus that will propagate to the rest of the cortical mantle (Ben-Ari and Cossart 2000; Ben-Ari and Gho 1988; Robinson and Deadwyler 1981). The mechanisms underlying these effects have been in part elucidated. Kainate activates high-affinity kainate receptors that are highly expressed on CA3 pyramidal neurons and their mossy fiber synapses (Gaiarsa et al. 1994; Monaghan and Cotman 1982; Tremblay et al. 1985), leading to the generation of synchronized bursts via the recurrent collateral excitatory synapses that interconnect CA3 pyramidal neurons (Miles and Wong 1986; Smith et al. 1995).

Kainate receptors are, however, also enriched on interneurons (Bahn et al. 1994; Bureau et al. 1999; Mulle et al. 1998, 2000; Petralia et al. 1994) and the activation of these receptors (Cossart et al. 1998; Frerking et al. 1998) produces a massive excitation of interneurons and a dramatic increase of the tonic inhibitory drive that impinges on the principal cells. Because of the important role of GABAergic inhibition in preventing the generation of seizures, this action is somewhat paradoxical as it may exert an anti epileptic effect. The aim of the present study was therefore to examine the functional consequences of a selective activation of kainate receptors located on interneurons. We relied on the observation that GluR5-containing kainate receptors are enriched in interneurons (Bahn et al. 1994; Bureau et al. 1999; Mulle et al. 1998, 2000; Petralia et al. 1994) and that their activation by the relatively selective agonist (*RS*)-2-amino-3-(5-tert-butyl-3-hydroxy-4-isoxazolyl)propionic acid (ATPA) (Clarke et al. 1997) excites interneurons and *augments* the tonic GABAergic inhibition that impinges on pyramidal neurons (Cossart et al. 1998). We have used the in vitro intact neonatal hippocampus (Khalilov et al. 1997b) and a triple chamber that enables the two hippocampi and their connecting commissures to be in three independent chambers (Khalilov et al. 1999a). In this chamber, it is possible to apply a convulsive agent to one hippocampus and test whether a putative anti-epileptic agent prevents the propagation of the seizure to the other hippocampus. This enables to avoid possible interferences between convulsive and anti-epileptic agents.

METHODS

The intact preparation that we used as well as the triple chamber have been described elsewhere (Khalilov et al. 1997, 1999). We used the intact hippocampal formations (IIHFs) of neonatal (P6-P7) Wistar rats that were placed into a conventional fully submerged chamber and perfused with ACSF at 30–32°C at a rate of 8–10 ml/min. Whole cell recordings were performed with patch electrodes with a resistance of 5–8 MΩ when filled with solutions containing (in mM): 135 K-gluconate, 0.1 $CaCl_2$, 2 $MgCl_2$, 2 Na2ATP, 1 EGTA, and 10 HEPES, pH 7.25, ([Cl^-]in = 4.2 mM), pH 7.25, osmolarity, 280 mosm. Tungsten bipolar electrodes disposed in the stratum radiatum of CA3 area were used to evoke synaptic responses. All neurons were filled with dyes and reconstructed post hoc for identification.

Group measures are expressed as means ± SE, error bars also indicate SE. Statistical significance of differences was assessed with the Students *t*-test, the level of significance was set at $P < 0.05$. Drugs

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used were purchased from Cookson-Tocris [6-cyano-7-nitroquinoxalene-2,3-dione (CNQX) and 2-amino-5-phosphonovaleric acid (D-APV)], Sigma (bicuculline, kainate, biocytin), and Alamone (TTX). ATPA was kindly provided by Lilly (Lilly Research Center).

RESULTS

ATPA excites interneurons and inhibits pyramidal neurons in the neonatal hippocampus

In an earlier study, we had demonstrated that the activation of GluR5-containing kainate receptors increased GABAergic inhibition in adult hippocampal slices (Cossart et al. 1998). We thus first tested whether this effect is also valid in neonatal neurons. We used P6–P7 intact preparations as the activation of GABAergic synapses generates at this age primarily a hyperpolarization (Ben-Ari et al, 1989). Bath application of ATPA (1 μM) produced opposite effects on cell excitability in simultaneously recorded CA1 interneurons and pyramidal neurons. In cell-attached recordings (Fig. 1, A and B), ATOA (1 μM) increased the action potential discharges of interneurons and reduced that of pyramidal neurons. In whole cell recordings at resting membrane potential, ATPA produced a hyperpolarization of pyramidal neurons ($x = 7.1 \pm 1.1$ mV, $n = 10$) in current-clamp conditions (Fig. 1*C*) and a dramatic increase of the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs; $x = 1{,}250.4 \pm 201.2\%$, $n = 5$). Therefore in neonatal neurons, the activation of GluR5 agonists induces an increase of the inhibitory drive via a depolarization of interneurons.

ATPA prevents the propagation of seizures from one hemisphere to the other

To determine the effects of ATPA on the propagation of epileptiform activities from one hemisphere to the other, field and patch-clamp recordings were made from both hemispheres. Bath application of high $[K^+]_o$ (7 mM), bicuculline (3 μM), or 4-AP (50 μM) to one hippocampus generated recurrent spontaneous and evoked inter-ictal episodes (Fig. 2). These are mediated by glutamatergic excitatory postsynaptic currents and are inward at V_m −45 mV(Fig. 2*C*). These events rapidly propagated to the contralateral hippocampus, (Fig. 2*D*,

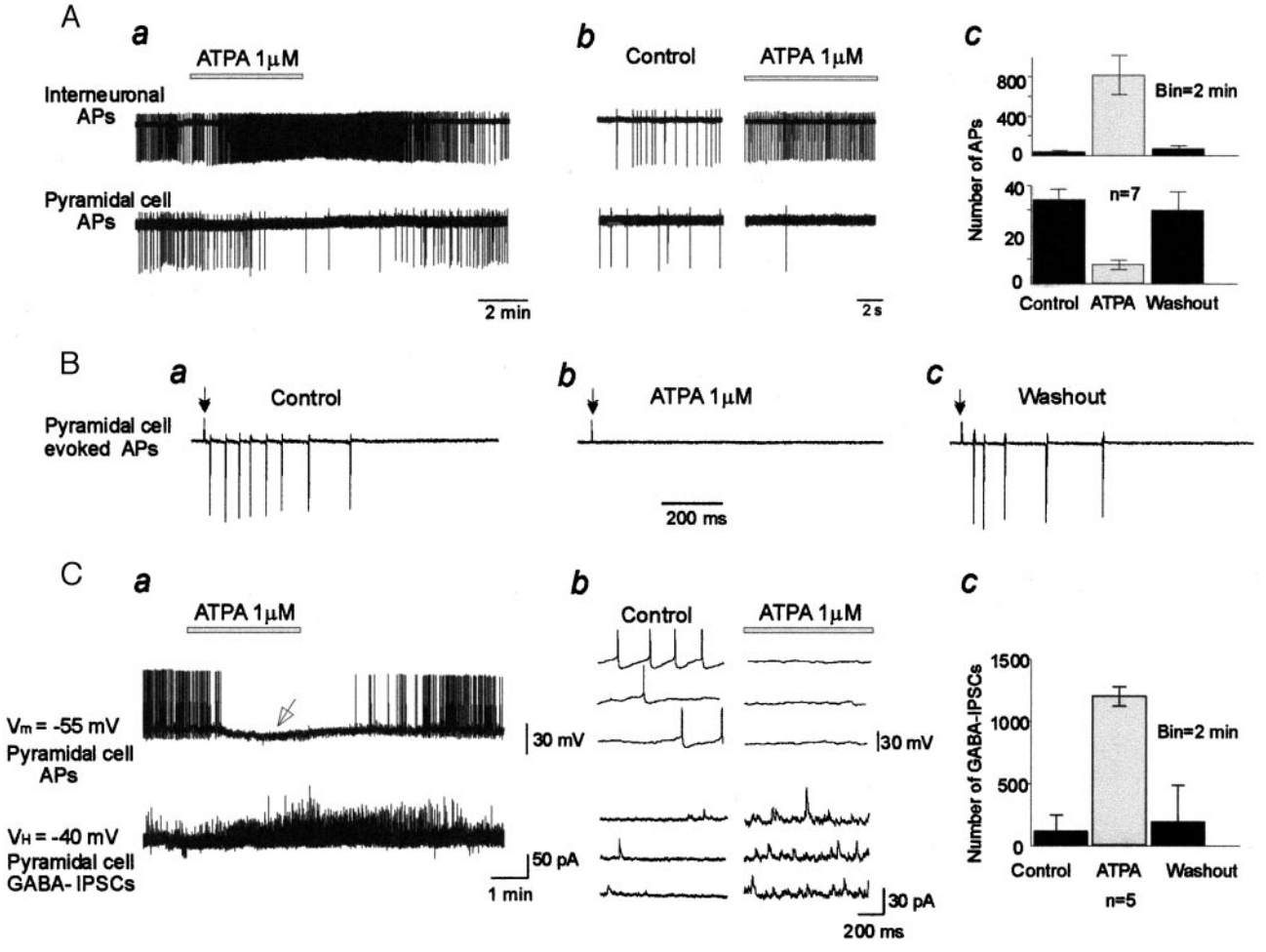

FIG. 1. (*RS*)-2-amino-3-(5-tert-butyl-3-hydroxy-4-isoxazolyl)propionic acid (ATPA) excites interneurones and inhibits pyramidal cells. *A*: paired recordings in the cell attached configuration from a P6 intact hippocampus in vitro. Note that ATPA produced a more than 40-fold increase of the frequency of action potentials in the interneuron and an almost full blockade of the discharge of the pyramidal neuron. *B*: electrical stimulation generated a barrage of action potentials in a pyramidal neuron. This was fully blocked by ATPA. *C*: paired recordings from 2 pyramidal neurons in whole cell configuration current clamp (*top*) and voltage clamp (*bottom*). Note that ATPA produced a hyperpolarization (*top*) and a massive increase of the frequency of the ongoing spontaneous inhibitory postsynaptic currents (sIPSCs; *bottom*). Quantitative data are presented in the right side of the figure.

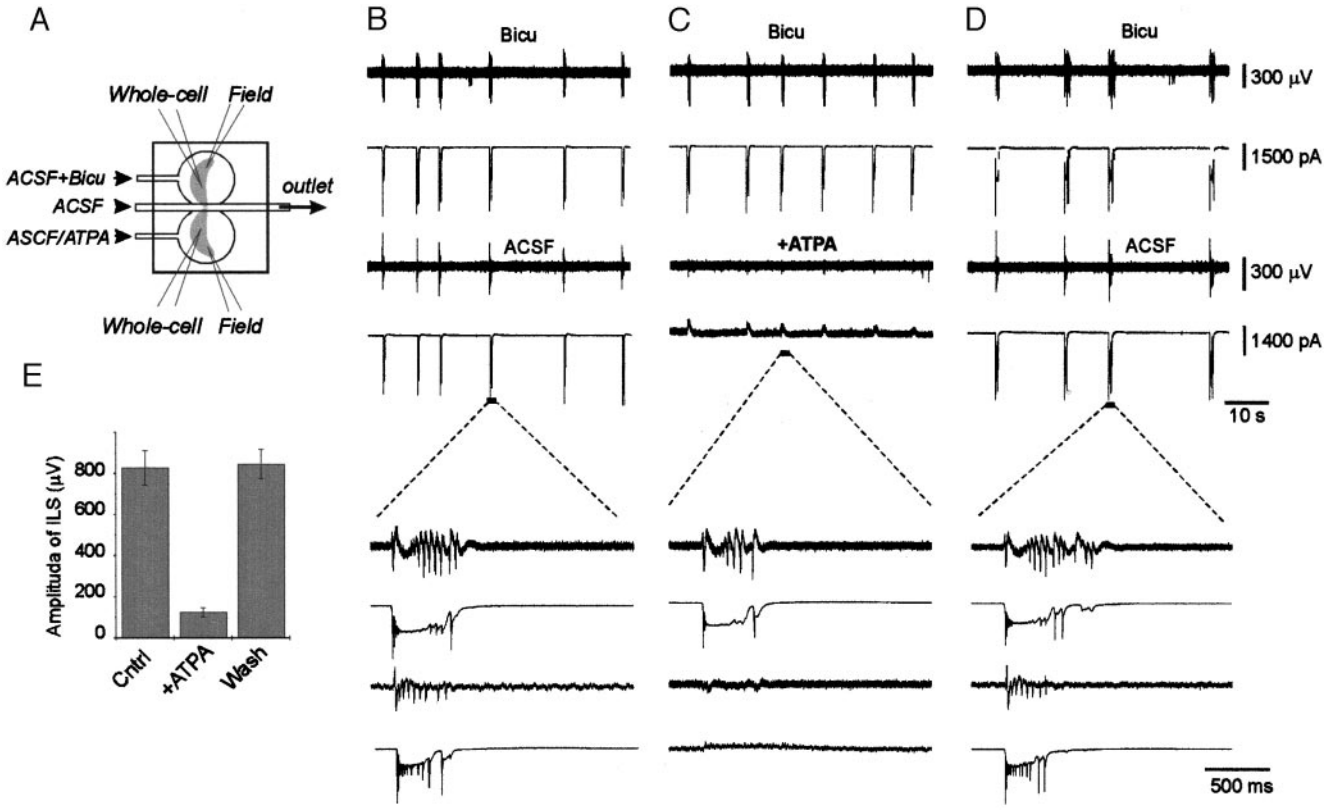

FIG. 2. ATPA prevents the propagation of seizures from 1 hemisphere to the other. *A*: triple chamber preparation, recordings include both field and whole cell recordings from both hippocampi. *B*: application of bicuculline (2 μM) to 1 hippocampus generated inter-ictal-like seizures (ILS) that propagated to the contralateral side. *C*: bicuculline-induced seizures failed to propagate to the contralateral hemisphere when ATPA (1 μM) was present. *D*: washing out of ATPA enabled the propagation of seizures to the contralateral hippocampus. *Bottom*: the same data at a faster display. Quantification of the effects of ATPA is shown in *E*.

mean latency of 25.3 ± 0.8 ms, $n = 24$). Applications of ATPA (1 μM) to the contralateral naïve hippocampus that did not receive the convulsive agent considerably reduced the propagation of seizures (Fig. 2*C*). Thus in most experiments (65% of the experiments, $n = 17$), it produced a complete blockade of epileptiform events in the remaining experiments it reversibly decreased both the amplitude and duration of inter-ictal events (66.4 ± 1.2 and 75 ± 5.0% of the control value, respectively, $n = 6$). During the application of ATPA, GABAergic currents were recorded in the contralateral hippocampus and glutamatergic ones in the treated hippocampus (Fig. 2*C*). On wash-out of ATPA, the seizures rapidly propagated to the opposite hippocampus (Fig. 2*D*). Bath applications of ATPA after the seizures had been generated in the contralateral hippocampus, similarly blocked the propagated seizures (not shown).

DISCUSSION

We show that the activation of GluR5-containing kainate receptors by ATPA prevents the propagation of epileptiform events from one hemisphere to the other. Although the exact mechanisms underlying these effects have not been determined, the powerful excitation of interneurons and the dramatic increase of the tonic inhibition that it generates most likely play an important role. However, ATPA is not entirely specific for GluR5-containing receptors (Paternain et al. 2000), and the present results are in disagreement with studies by Lerma and co-workers that suggest that kainate reduces inhibition in adult neurons (Ben-Ari and Cossart, 2000, 2001; Lerma 2001; Lerma et al. 2001). Also, the effects of ATPA on interneurons are reduced but not fully blocked by a selective KO of GluR5-containing receptors; a double KO of GluR5 and -6 is required to produce a full blockade of the actions of ATPA (Mulle et al. 2000). Finally, several interneurons—in particular in stratum radiatum—are not excited by ATPA (Cossart et al.) suggesting that other mechanisms may be involved in the actions of kainate. In spite of this, our data are best explained by a direct excitation of interneurons and a reduction of the excitability in principal cells. Our observations should provide a novel approach to reduce the excitability of principal cells in a cortical network and possibly prevent seizure generation. The use of the triple chamber provides a unique opportunity to test these actions without the possible interferences between the convulsive and anti-epileptic actions of an agent.

Several features of the actions of ATPA, kainate—or other activators of kainate receptors located on interneurons—are particularly useful to consider in relation to its suggested anti-epileptic properties. First, the excitation of CA1 interneurons by these agents is specific because similar applications of kainate or ATPA do not excite CA1 pyramidal neurons (Cossart et al. 1998). Second, this effect is dramatic and persistent, it is associated with an 8- to 10-fold increase of the frequency of GABAergic IPSCs in pyramidal neurons that can persist for long durations (Cossart et al. 1998; present study). Third, the

synaptic input activated by kainate receptors in interneurons is quite powerful as it readily generates action potentials and produces an important increase in the excitability of interneurons (Cossart et al. 1998; present study). Nevertheless, it is important to stress that other actions of ATPA could contribute to the observed effects. Recent studies suggest that kainate receptor activation could also reduce glutamate release by a presynaptic mechanism (Contractor et al. 2000; Frerking et al. 2001). Further studies are required to determine the contributions of these actions to the anti-epileptic actions of GluR5 agonists.

Our suggestion of paradoxical anti epileptic effects of a kainate receptor agonist is not incompatible with the epileptogenic effects of kainate. Kainate exerts a multitude of actions on different types of neurons and different channels. The result—the seizure—is the consequence of these diverse actions. Thus seizures are generated in the adult hippocampus primarily because of the activation of the mossy fiber synapses that are enriched with the GluR6-containing kainate receptors. Both the knock-out of the GluR6 genes (Mulle et al. 1998) and the selective lesion of the mossy fibers by neonatal irradiation prevent the effects of kainate. Also, GABAergic inhibition is not abolished in epileptic animals; there is rather a reduction of tonic inhibition restricted to the dendrites of pyramidal neurons—as a result of the degeneration of a subpopulation of interneurons during the initial status epilepticus. In contrast, tonic inhibition is increased in the somata of the principal cells raising the possibility of a paradoxical increase of inhibition in the epileptic circuit (Cossart et al. 1998, 2001). As interneurons are capable of high-frequency discharge, we suggest that the activation by GluR5 agonists of surviving interneurons may in part replace the missing inhibitory drive. Preliminary experiments suggest that the actions of ATPA are preserved in neurons recorded in slices obtained from epileptic rats (Hirsch, unpublished observations).

Further studies are also required to determine the maturation of the actions of ATPA. Indeed early during development, GABA provides most of the excitatory drive because of a different chloride gradient (Ben-Ari 2000; Ben-Ari et al. 1989). In our studies, ATPA prevented the propagation of seizures even when GABA generated a depolarization in the recorded neuron (not shown). This is best explained by the dual excitatory and shunting actions of GABA shown in neonatal hippocampal neurons (Khalilov et al. 1999a). Future studies will also have to compare the maturation of the epileptogenic actions of kainate (see Khalilov et al. 1999b) to the development of the effects of ATPA. Immuno-cytochemical data suggest that interneurons possess GluR5 mRNA already at birth (Bahn et al. 1994).

In conclusion, our results suggest that the activation of GluR5-containing kainate receptors can prevent the propagation of seizure from one hemisphere to the other. Kainate will generate seizure but at the same time augment the excitatory input to interneurons and thus lead to an increased inhibition. The observation that these actions may be mediated by different subunits will enable to develop selective drugs that predominantly act to reduce seizures. The use of the newly developed triple chamber provides a unique possibility to record from both hemispheres and determine the effects of various procedures and agents on the generation and propagation of activities from one hemisphere to the other.

This work was supported by Institut National de la Santé et de la Recherche Médicale, the French League against Epilepsy (LFCE), and a grant from the Foundation Electricity of France to I. Khalilov.

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